

Establishing positional information through gradient dynamics

A lesson from the Hedgehog signaling pathway

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A long standing question in developmental biology is how morphogen gradients establish positional information during development. Although the existence of gradients and their role in developmental patterning is no longer in doubt, the ability of cells to respond to different morphogen concentrations has been controversial. In the *Drosophila* wing disc, Hedgehog (Hh) forms a concentration gradient along the anterior-posterior axis and establishes at least three different gene expression patterns. In a recent study, we challenged the prevailing idea that Hh establishes positional information in a dose-dependent manner and proposed a model in which dynamics of the gradient, resulting from the Hh gene network architecture, determines pattern formation in the wing disc. In this Extra View, we discuss further the methodology used in this study, highlight differences between this and other models of developmental patterning, and also present some questions that remain to be answered in this system.

Introduction

In a developing organism, cells require information about their relative position in order to function and differentiate appropriately. Despite the discovery that key signaling pathways act as organizers of pattern formation in several systems, the details of how this positional information is distributed, processed and interpreted by cells in a developing field remain little understood. One way by which cells may acquire their spatial coordinates is by measuring the concentration of signaling

molecules called morphogens.¹ The classical morphogen model states that different concentrations within the gradient correspond to different “positional values” which determine the location of specific gene expression patterns.² A key idea of the classical morphogen model is the existence of concentration thresholds in the gradient that correspond to well-defined boundaries of gene expression. Several studies recognized that the classical morphogen model by itself may not be sufficient to account for the precision and reproducibility often observed in developmental patterning and proposed additional properties to the model that include feedback control,^{3–5} cell-to-cell interactions,^{6,7} and the integration of the signal over time.^{4,8} However, the idea that morphogens initiate the process of developmental pattern formation in a dose-dependent manner prevails in the current literature.^{9–11}

Hedgehog (Hh) organizes patterning along the anterior-posterior (AP) axis of the *Drosophila* wing disc; it functions upstream of other signaling pathways such as TGF β and EGFR,^{12,13} and ectopic Hh expression causes pattern duplications and ectopic tissue in the adult.¹⁴ Furthermore, at least three different domains of gene expression are specified directly by Hh signaling.^{14,15} These observations have supported the view that Hh functions in the *Drosophila* wing disc as a classical morphogen.^{14,16} However, a direct causal relationship between the boundaries of gene expression patterns and Hh concentration thresholds has never been demonstrated.

Key words: Hedgehog, developmental patterning, morphogen, dynamics, mathematical modeling

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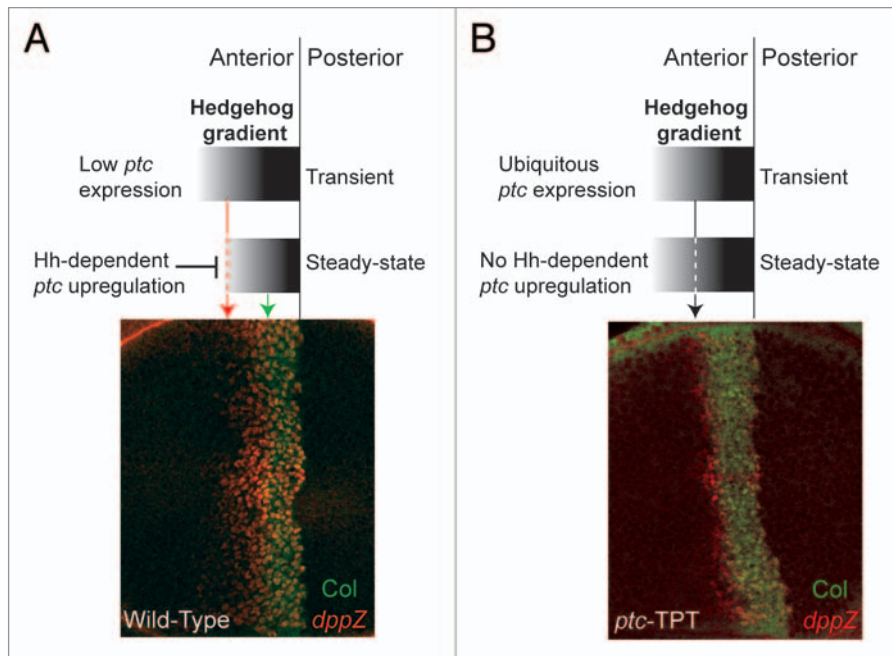


Figure 1. Hh gradient dynamics patterns the Drosophila wing disc. (A) In wild-type discs, low levels of the Ptc receptor allow the Hh gradient to expand and define a transient domain of signaling activity. Later on, *ptc* is upregulated in response to Hh signaling and results in ligand sequestration followed by a posterior refinement of the Hh gradient. This temporal shift (or overshoot) of the gradient differentiates the location of the anterior boundaries of *dpp* (red arrow) from other Hh target genes such as *col* (green arrow). In principle, every target gene responds to approximately the same dose of signal activation, but only *dpp* expression is maintained in the domain defined by the transient gradient. (B) In *ptc* mutant discs carrying a transgene that ubiquitously expresses *ptc* (*ptc*-TPT), the lack of Hh-dependent *ptc* upregulation causes the Hh gradient not to exhibit the wild-type overshoot dynamics as in (A). As a consequence, the patterns of *col* and *dpp* approximately share the same anterior boundary (black arrow). This model does not require multiple concentration thresholds to establish different patterns of gene expression. Photos were adapted from ref. 17 and depict a wild-type (A) or a *ptc*-TPT (B) third instar wing discs carrying a *dpp*-lacZ reporter (*dpp10638*) and immunostained using anti-Col (green) and anti- β -galactosidase (red) antibodies.

In a recent study,¹⁷ we initiated an analysis of Hh-dependent patterning by using mathematical modeling to guide our idea of how patterns of gene expression form along the AP boundary of the Drosophila wing disc. Our steady-state analysis of the model suggested that Hh signaling can only support two states (ON and OFF) and formed the basis for the hypothesis that the interpretation of positional information might depend on the dynamics of the Hh gradient. Specifically, a single Hh concentration switch (ON/OFF) may be sufficient to provide positional information in this system when the history of gradient formation is factored in. In support of this idea, our in vivo experimental findings suggested that the Hh gradient transiently expands farther from the AP boundary with respect to its steady-state position.

We propose that this change in the Hh distribution—or “overshoot”—is a consequence of Hh-dependent upregulation of the receptor Patched (Ptc) and causes some cells to be transiently exposed to Hh signaling. Furthermore, we found that this dynamic behavior of the gradient is required to define different patterns of gene expression (Fig. 1). In particular, cells that maintain sustained levels of Hh signaling express the Hh target genes *engrailed* (*en*), *ptc*, *collier* (*col*) and *decapentaplegic* (*dpp*). However, cells transiently exposed to Hh that lose activity of the signal due to refinement of the gradient will maintain *dpp* expression, but not the expression of *en*, *col* or *ptc*. This selective ‘memory’ of *dpp* expression distinguishes cells transiently exposed to Hh from cells that were never exposed to it. Here, we analyze the methodological and biological

contributions of this study and highlight open questions about this dynamic model of developmental patterning.

Mathematical Modeling as a Hypotheses-Generating Tool

Mathematical modeling and theoretical biology have led efforts to investigate the question of how patterns emerge during development.^{18–20} Today, there is no doubt that the interplay between theory and experiment has significantly advanced our current understanding of developmental processes.^{21,22} A common approach has been to use available experimental data to devise mathematical models that can be used as predictors of experimental results (Fig. 2A). Typically, these models are then used to explore properties of the system that are not easily exploited by experimentation. Alternatively, mathematical models can be formulated to discriminate between different interpretations of an experiment (Fig. 2B). Although the use of mathematical models as *predictors* (Fig. 2A) or *interpreters* (Fig. 2B) of experimental data has often resulted in important contributions to developmental biology, they have also been subject of skepticism from experimentalists. A major criticism to these approaches is that conclusions arise from the models themselves, and as such, depend on the details of their mathematical formulation and their accuracy to represent the biological phenomenon.

In our recent study of the interpretation of Hh signaling,¹⁷ we used mathematical modeling as a tool to formulate hypotheses that could be tested through direct experimentation (Fig. 2C). Importantly, these hypotheses would not be straightforward to propose without the mathematical model. Specifically, we modeled the Hh signaling pathway using a system of partial differential equations and found that under certain assumptions (i.e., the value of a critical parameter), interpretation of the Hh gradient in a concentration-dependent manner was not possible. Furthermore, the model suggested that the formation of the gradient follows some unusual dynamics due to a property of the gene network architecture associated with the Hh signaling pathway,

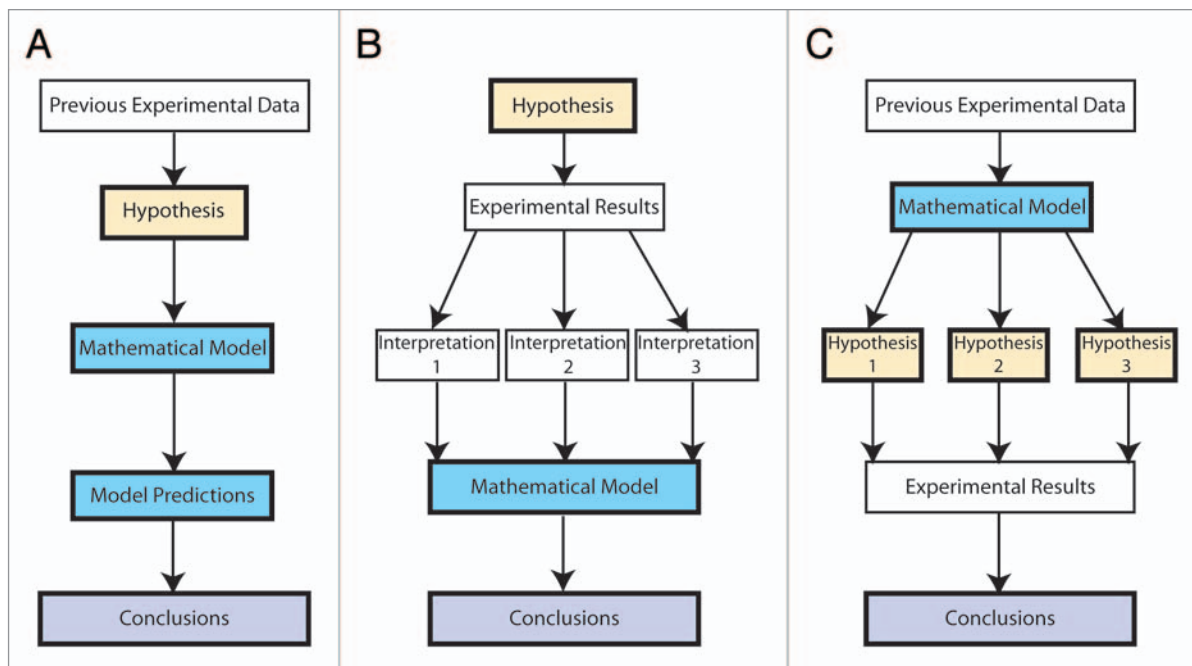


Figure 2. Different methodologies for using mathematical modeling in biological research. (A) The mathematical model may be used as a *predictor* of new experimental results. This approach is typically used by theoreticians to build mathematical models based on available experimental data. (B) A mathematical model can also be used as an *interpreter* of experimental data. In this case, the model is employed to test the feasibility of different interpretations of an experiment. (C) Mathematical modeling as a *hypotheses-generating tool*. In this approach, the mathematical model is used to propose different hypotheses, but does not favor any particular one. Unlike the methodologies depicted in (A) or (B), in (C) conclusions are exclusively derived from experimental data.

namely, that the Hh receptor and antagonist, *ptc*, was transcriptionally upregulated in response to Hh signaling. The gradient initially expands due to low Ptc levels, but then retracts as a result of Ptc accumulation which leads to the sequestration and degradation of free extracellular Hh (Fig. 1A). Moreover, we showed experimentally that if Hh-dependent *ptc* upregulation is impaired then the signal fails to establish different domains of gene expression (Fig. 1B). Thus, the model did not predict that the dynamics of the gradient were required for the interpretation of the signal, but rather prompted us to investigate it. In contrast to other modeling approaches that have utilized mathematical models as predictors of unknown data (Fig. 2A) or to interpret unclear experiments (Fig. 2B), in our study the model was used as a motor to propose non-trivial hypotheses (Fig. 2C). Our approach is somewhat similar to model-based experimental design strategies in which mathematical models are used to define possible experiments that can be performed. Although these approaches have become widely used in systems

biology in the post-genomic era,^{23,24} our approach—to employ mathematical modeling as a tool to guide experimental research—is not common in the context of developmental biology.

A Network Architecture-Based Model of Developmental Patterning

The most important conceptual contribution of our recent work on Hh signaling, in our opinion, is the idea that the shape of the gradient is not the major factor contributing to pattern formation in this system. It is widely recognized that developmental patterning is tightly controlled by feedback components inherent within the gene regulatory network of the system.²⁵ These feedback interactions have been shown to be essential for generating sharp boundaries of gene expression and to ensure reproducibility and precision under genetic or environmental perturbations. However, most models of morphogen-mediated developmental patterning are built under the main hypothesis that pattern formation is a function of

the morphogen concentration profiles. In particular, changes in patterning are usually directly associated with changes in the morphogen distribution and properties such as precision, robustness or size-dependent scaling are generally studied assuming that the shape of the morphogen gradient is the predominant factor.^{26–29} Our study suggests that Hh-dependent patterning in the *Drosophila* wing disc depends on temporal changes of the morphogen profile but, unlike the classical morphogen model, it does not primarily depend on concentration thresholds defined by the distribution of the gradient; instead, patterning is controlled directly by the architecture associated with the Hh gene network, particularly by the feedback that results from Hh-dependent *ptc* upregulation and Ptc-dependent ligand sequestration. Therefore, our model is in agreement with a recent study³⁰ that supports the idea that pattern formation is inherent within the gene regulatory network of the system and concludes that the shape of the Hh concentration profile is not the primary source of positional information.

Open Questions Regarding Hh Patterning in the Wing Disc

Although our model can explain the experimental data regarding the emergence of multiple patterns of gene expression in response to Hh in the *Drosophila* wing disc, many questions do remain unanswered. In this section, we briefly present some aspects that require further attention in the future.

Our study provides evidence for the existence of a Hh gradient overshoot upon reinitialization of the gradient using a temperature-sensitive *hh* allele, but when such a dynamic shift in the gradient occurs during normal development remains to be identified. Alternatively, it is also possible that the overshoot occurs multiple times in wing disc development. Such oscillations in the range of the signal may occur if Hh-dependent Ptc upregulation becomes sufficiently strong so that Hh signaling is repressed completely, and thus that expression of *ptc* is interrupted—allowing for multiple rounds of Hh gradient expansion and refinement. However, this periodic behavior of Ptc expression has not been reported and, furthermore, we suggest that such regulation is unlikely to occur in a synchronized manner. These important aspects will require direct temporal examination of Hh gradient formation and Ptc expression in living tissues over a long period of time, but this remains technically challenging.

Another important problem that will require further investigation is how this model accommodates tissue growth. In particular, if cells that experience a transient Hh signaling retain *dpp* expression by some ‘memory’ mechanism, whether or not this is retained after cell division is still in question. Our data show that the time-scale of the overshoot (~6 hours) is shorter than the average cellular proliferation rate in the wing disc during the third instar (~8.5 hours³¹), therefore the dynamics of the gradient should not be directly affected by tissue growth. However, it remains unclear why all the cells derived from *dpp*-expressing progenitors do not retain *dpp* expression; a fraction of cells that are sufficiently close to the AP boundary (where Hh signaling

is ON) may end up located farther away from it as a result of tissue growth (where Hh signaling is OFF). One possible explanation is that cells expressing *dpp* maintain their relative position in the wing disc as a result of cell affinity, but their progeny eventually lose the ability to maintain *dpp* expression and are pushed away from the anterior-posterior boundary. In fact, the hypothesis that *dpp*-expressing cells attempt to remain together during wing disc development is supported by a study that suggest that *dpp*-expressing cells may regulate a cell adhesion molecule that is necessary to avoid intermixing of anterior and posterior cells.³² However, it is unclear if the progenitors of *dpp*-expressing cells that are no longer exposed to Hh would lose their ability to maintain *dpp* expression. In summary, the relationship between patterns and growth and specifically, how *dpp* ‘memory’ is affected by cell proliferation deserves further investigation as well.

Our model of Hh-dependent patterning in the *Drosophila* wing disc primarily depends on a particular gene network architecture, rather than on Hh concentration thresholds. Numerous studies in different developmental contexts have revealed that the Hh signaling gene network architecture is largely conserved from flies to humans. In particular, Hh-dependent *ptc* upregulation is a common feature in all the systems studied so far. Thus, an exciting question for the future is whether similar models of pattern formation hold for systems with equivalent network architectures or the principles of developmental pattern formation evolved despite the conservation of gene network topologies. Recent data from the vertebrate neural tube suggest that cells determine their fate by integrating the strength of Hh signaling over time,³³ while another study in the same system reported that some positional information is lost when *ptc* is not upregulated in response to Hh signaling.³⁴ These results are in close agreement with our model of Hh patterning in the *Drosophila* wing disc,¹⁷ but additional studies will reveal if developmental patterning in other Hh-dependent systems employs similar principles.

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